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Photo-enhancement of Cr(VI) reduction by fungal biomass of *Neurospora crassa*

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ABSTRACT

Various organisms such as fungus are capable of reducing Cr(VI) to less toxic Cr(III). However, light-induced Cr(VI) reduction by fungus is less reported and needs to be explored since anthropogenic or natural activities may bring these two reactants into a sunlit environment. In this study, the interactions and reaction mechanisms of Cr(VI) on a model fungus, *Neurospora crassa*, were evaluated in the presence or absence of light. The influence of ferric ion, a widely distributed metal, on Cr(VI) reduction by the fungus was also investigated under illumination. The results show that 20–54% of added Cr(VI) (96.2 μM) was removed by 1 g of dead fungal biomass (i.e., 1–2.7 mg Cr(VI) reduction by 1 g biomass) at pH 1–3, after 6 h reaction in the dark. However, 96.2 μM Cr(VI) disappeared completely (i.e., 5 mg Cr(VI) reduction by 1 g biomass) under the same reaction time and experimental conditions when light was present. The rapid disappearance of Cr(VI) in solution was due to the reduction of Cr(VI) by the excited biomass upon light absorption, and the rates of redox reactions increased with a decrease at pH. Cr(VI) reduction could be further increased with the addition of 89.5 μM Fe(III) because the formation of Fe(II) from the photolysis of Fe–organic complexes enhanced Cr(VI) reduction. Spectroscopic studies indicated that the amide, –NH, and carboxyl groups of *N. c.*-biomass may be responsible for initiating Cr(VI) reduction; comparatively, the cyclo-carbons of chitin, glucan, and their derivatives were more persistent to the oxidation by Cr(VI). Accordingly, fungi containing high amount of carboxyl, amide, and –NH groups may be preferable as efficient reductants for scavenging Cr(VI) from environment. Upon the absorption of a renewable light source, Cr(VI) could be converted rapidly by the biomaterials to the less toxic Cr(III).

1. Introduction

Chromium exists not only in natural resources, but is also found in many wastes released from various industrial activities such as electroplating effluents, metal-finishing waste streams, and sewages and wastewater treatment plant discharges [1]. The oxidation states of Cr vary from 2^- to 6^+; however, tri- and hexavalent forms of Cr predominate in the environment [2]. Trivalent Cr (Cr(III)) is less toxic and less mobile, and is considered an essential micronutrient for human beings [3]. Conversely, Cr(VI) is toxic to both plants and animals [4]. In particular, exposure to Cr(VI) may increase the risk of cancer or lead to the development of hypersensitivity in the skin or respiratory system [5]. Therefore, efforts to eliminate Cr(VI) from the environment are necessary to prevent the deleterious impact of Cr(VI) on ecosystems and public health. Treatment of Cr(VI)-contaminated wastewater is usually accomplished by the addition of abiotic adsorbents and chemical reductants to decrease its concentrations [6]. However, these reagents may add extra burdens to the environment or cause additional hazards to ecosystems once they are used and disposed of, if they contain residual Cr(VI) [7]. Recently, biomasses derived from bacteria, yeast, seaweed, algae and higher plants have been confirmed to be effective bio-adsorbents for Cr(VI) removal [8–13]. These studies mainly emphasized the influences of pH, ionic strength, other metals, and solution chemistry on the immobilization of Cr(VI) onto raw or chemically modified biosorbents. The interactive mechanisms of Cr(VI) and surface functional groups of biosorbents were not investigated as fully. If the activated sites of biosorbents involved in Cr(VI) bindings is to be understood, the differences in various biomaterials for the quantitative and kinetic removal of Cr(VI) must be systemically explained. This might allow reliable selection of biomaterials for Cr(VI) removal without extensive investigation prior to their application.

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Besides the biomaterials mentioned previously, Cr(VI) removal by dead fungal biomass have also received much attention. Prakasham et al. [14], and Bai and Abraham [15]) suggested that Cr(VI) was removed through a mechanism of "anionic adsorption" on the specific functional groups of biomass. However, Park et al. [12] reported that bio-adsorption was not the only process that attributed to Cr(VI) removal by the biomass in an acidic solution. They found that Cr(VI) could be reduced to Cr(III), which was subsequently released to solution or bound partly to the biomass. These studies demonstrated that the natural-occurring biomasses may substitute as a reductant for Cr(VI). Nonetheless, kinetic reduction of Cr(VI) by biomaterials such as humic substances and rice husks is extremely slow even in an acidic solution in which the thermodynamic reduction of Cr(VI) is feasible [16,17]. Although some natural biomaterials such as seaweeds, pine needles, and specific fungi may remove tens to hundreds of milligrams per liters of Cr(VI) in hours, high biomaterial concentrations (e.g., 5–10 g/L) with a high temperature are required to achieve the rapid Cr(VI) reduction [13,17,18].

Previous studies suggested that Cr(VI) reduction by organic matters such as humic substances and low molecular weight organic compounds could be greatly enhanced in the presence of light [16,19] and ferric ions [20]. The photo-induced pathway can be considered a shortcut for Cr(VI) reduction in a sunlit environment containing various organics and ferric ions. Since the biomaterial such as fungus derived from industrial effluents or surface run-off may move into the water bodies where receive sunlight, its contribution to Cr(VI) reduction should not be ignored. Considering the abundance of fungus with multifunctional carbon groups, the biomaterial may serve as an efficient bio-reductant for Cr(VI) under illumination. Besides, the specific sites on fungus involved in Cr(VI) reduction may be more readily clarified than that on humic substances because the fungus contains only aliphatic carbons; however, both aromatic and aliphatic domains are present in the structures of humic substances. Therefore, the objectives of this study were to evaluate the efficiency of Cr(VI) removal in the presence of light by a dead fungal biomass, using *Neurospora crassa* as a model biomaterial because it is readily cultured and grown. The influences of irradiation time and pH on Cr(VI) reduction by the dead fungal biomass in the presence or absence of Fe(III) was investigated. In addition, the possible functional groups involved in the process of Cr(VI) removal, either in the dark or exposure to the light, were identified and systemically investigated.

2. Materials and methods

2.1. Preparation of the dead fungal biomass

The strain used in this study was *N. crassa* 74A (denoted as *N. c.*-biomass). The liquid growth medium (Vogel's salts) was autoclaved at 121 °C for 30 min. Spores from a mature slant of *N. c.*-biomass were inoculated in a Erlenmayer flask (125 mL) containing 50 mL Vogel's salts under sterile conditions. The *N. c.*-biomass in the flask was cultured for 2 days at 26 °C on a rotary shaker (150 rpm). After 2 days, the biomass was collected, followed by filtration to remove the growth medium. The resulting biomass was washed several times thoroughly with sterile water and then freeze-dried for 24 h. The dried biomass was stored in desiccators before use.

2.2. Cr(VI) removal by biomass

Cr(VI) removal by *N. c.*-biomass was conducted in a water-jacketed reaction vessel, surrounding by a 100 W medium-pressure mercury UV lamp inserted in a borosilicate well to filter out $\lambda \leq 290$ nm. The lamp emits mostly in the range 310–1000 nm, with the most intense lines at 303, 313, 366, 405, 436, 546, and 578 nm. The average light intensity of $2.55 \times 10^{-4} \pm 1.39 \times 10^{-5}$ einstein s^{-1} L^{-1} ($n = 18$) was obtained in the reaction vessel based on an actinometric technique [21]. The reaction vessel and the borosilicate well were both connected to a circulating water bath to maintain their temperature at 24.5 ± 0.5 °C. The *N. c.*-biomass was suspended in a solution with 0.01 M KCl for 1 h, followed by the addition of 100 mg L^{-1} Cr(VI) stock solution prepared by dissolution of $K_2Cr_2O_7$. The volume of the mixture was brought to 250 mL, and the solution pH was adjusted and maintained at 1–3. No buffer was used because the change of pH was less than 0.03 during the experiment. The final concentrations of *N. c.*-biomass and Cr(VI) were 1 g L^{-1} and 96.2 μM, respectively. In another set of experiment, a final concentration of 89.5 μM Fe(III) as $FeCl_3$ was added into the solution with the same amount of biomass and Cr(VI). Light control and dark control experiments were also conducted at these experimental conditions in the absence of *N. c.*-biomass. At each specific reaction time, suspensions were extracted and passed through a 0.2 μm pore-sized membrane filter. Cr(VI) concentration was determined using a UV/vis spectrophotometer (Variance Cary 50) at 540 nm after reaction with an 1,5-diphenylcarbazide indicator. Total chromium was determined by atomic absorption spectroscopy (AAS, HITACHI Z-2000) at λ = 359.3 nm. Each experiment was conducted in triplicate.

To examine whether Cr(VI) could be adsorbed by biomass without being reduced, 3 mL of suspension was withdrawn and transferred to a 12-mL 0.1 M K_2HPO_4/KH_2PO_4 buffer solution (ca. pH 7) to desorb Cr(VI) from biomass for 15 min [22], a time in which preliminary experiments had showed that desorption of adsorbed Cr(VI) could reach maximum. This set of experiment was always conducted in the dark. The amount of adsorbed Cr(VI) on the bio-adsorbent was obtained by taking the difference between Cr(VI) concentrations before and after the addition of KH_2PO_4.

2.3. Characterization of fungal biomass

The elemental contents (C, H, N, and O) of the raw biomass without Cr(VI) treatment were investigated by a Heraeus CHNOS Rapid F002 Elemental Analyzer. ^{13}C CP-MAS NMR spectra and infrared spectra of the raw biomass and the Cr(VI)-loaded biomass treated with 100 mg L^{-1} at pH 1.0 for 20 h in the presence of UV light, were obtained using a Bruker DSX400WB NMR spectrometer and Fourier Transform Infrared (FTIR) Spectrometer (Thermo Nicolet NEXUS), respectively. The raw material was also pre-treated with an acidic solution (pH 1) for 20 h under illumination except Cr(VI) was not present. ^{13}C CP-MAS NMR spectra of the samples were collected under a magic angle spinning (MAS) rate of 6.5 kHz. For each scan, a pulse with a carrier frequency of 100 MHz was applied and the recycle time was 1.5 s. The number of scans was 2168 for each spectrum. For FTIR analysis, a precise amount of biomass (1 mg) was first mixed with KBr (0.2 g), and the mixture was grounded and compressed into a translucent sample disk. FTIR spectra in the range of 4400–400 cm^{-1} were obtained by co-addition of 64 individual scans with an optical resolution of 4 cm^{-1}.

3. Results and discussion

3.1. Cr(VI) removal by N. c.-biomass

Cr(VI) removal on *N. c.*-biomass proceeded rapidly during the initial several minutes, followed by a slight decrease in Cr(VI) concentration with a prolonged reaction time (Fig. 1a). Since dark control experiment showed that Cr(VI) remained constant in the absence of *N. c.*-biomass, the decrease in Cr(VI) should result from the interactions of Cr(VI) with biomass. The reaction pattern is

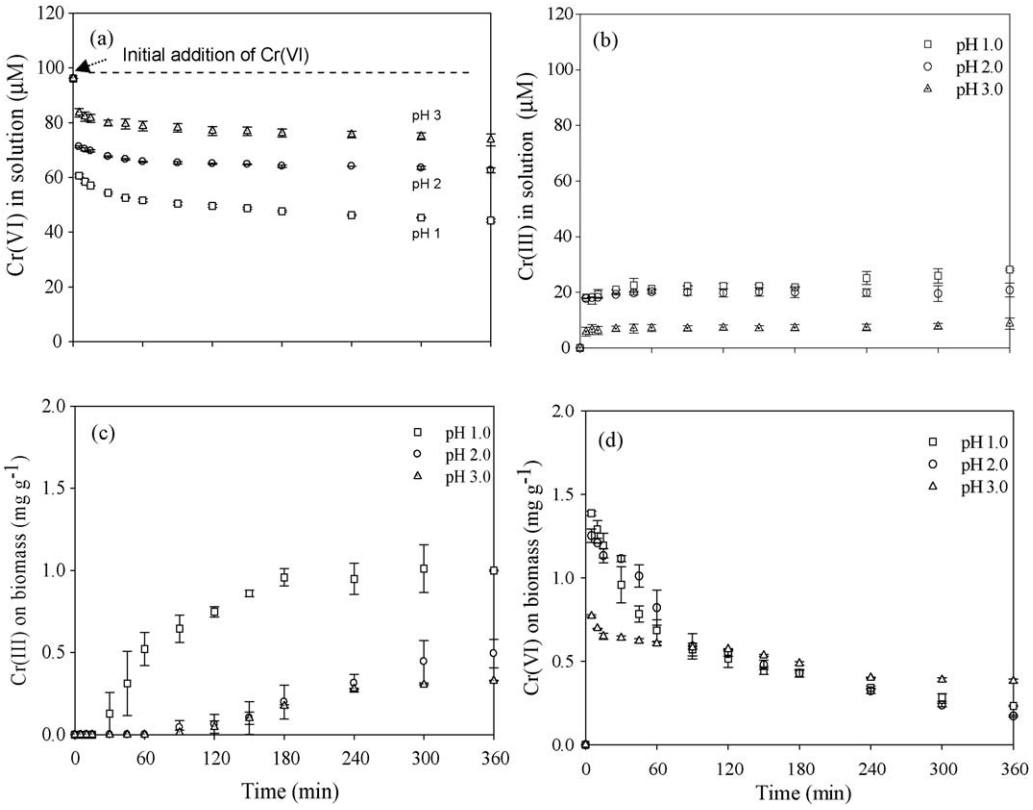

Fig. 1. Removal of 96.2 μM Cr(VI) by a dead *N. c.* fungal biomass (1 g L^{-1}) at pH 1–3 in the dark. The disappearances of Cr(VI) in solution and biomass are shown in (a and d), respectively. The formation of Cr(III) in solution and in biomass are shown in (b and c), respectively.

independent of solution pH; however, Cr(VI) removal tended to be greater with a decrease in pH, as shown by the increase in the first-order rate constants from 0.0003 to 0.0010 min^{-1} as the pH decreased from 3 to 1 (Table 1). The favorability of low pH for Cr(VI) removal may be due to the enhancement of both reduction and adsorption of Cr(VI) under a proton-rich environment. Cr(VI) reduction by organic reductants such as biomaterials accompanied with proton consumption has been previously reported (Eq. (1)) [12].

$$HCrO_4^- + 7H^+ + 3e^- \text{ (from biomaterials)} \rightarrow Cr^{3+} + 4H_2O + \text{oxidized biomaterials} \quad (1)$$

This reaction would lead to an elevation in solution pH; however, no discernible changes in pH were observed in the current study. Due to the low reduction stoichiometry of Cr(VI) (<50 μM after 360 min reaction even at pH 1, Fig. 1a), only were a small amount of protons consumed in the system, which may lead to the indiscernible changes in pH. Accompanied the gradual decrease in Cr(VI), an increase in Cr(III) was observed in the solution (Fig. 1b, obtained by Eq. (2)) and on the biomass (Fig. 1c, obtained by Eq. (3))

$$Cr(III)_{\text{in solution}} = Cr_{\text{total Cr in solution, measured by AAS}} - Cr(VI)_{\text{in solution, measured by DPC}} \quad (2)$$

$$Cr(III)_{\text{on biomass}} = Cr_{\text{initial Cr}} - Cr_{\text{total Cr in solution, measured by AAS}} - Cr(VI)_{\text{on biomass, determined by P extraction}} \quad (3)$$

The appearance of Cr(III) provide direct evidence that Cr(VI) was reduced to Cr(III) by *N. c.*-biomass. The reduction of Cr(VI) to Cr(III) may be through direct or indirect "contacting-reduction" pathways on the biomass, as proposed by Park et al. [17]. The "contacting-reduction" pathways emphasize the importance of solid surfaces of biomass involved in the Cr(VI) reduction.

As mentioned previously, adsorption of Cr(VI) on biomass was another factor in the acceleration of Cr(VI) removal at low pH. The surfaces of *N. c.*-biomass, having a point zero charge (PZC) at pH 6.55 (Table 1), are positively charged at pH ≤3, and the density of positive charges increased with a decrease in solution pH. Positively charged surfaces are more favorable for the adsorption of anions, because of electrostatic attraction (Fig. 1d) [23].

Table 1
First-order rate constants (k) for the removal of 96.2 μM Cr(VI) by dead fungal biomass in the presence or absence of 89.5 μM Fe(III) and UV light at pH 1–3.

	pH	No Fe(III)		With Fe(III)	
		k (min^{-1})	R^2	k (min^{-1})	R^2
Dark	1.0	0.0010 ± 1.5E−04	0.85	0.0009	0.86
	2.0	0.0005	0.84	0.0007	0.86
	3.0	0.0003 ± 1.5E−04	0.84	0.0002	0.83
Light	1.0	0.0231 ± 2.5E−03	0.97	0.0920 ± 1.1E−02	0.95
	2.0	0.0213 ± 1.7E−03	0.98	0.0738 ± 1.0E−03	0.97
	3.0	0.0134 ± 2.5E−04	0.99	0.0235 ± 2.1E−03	0.98

3.2. Enhancement of Cr(VI) removal on N. c.-biomass under illumination

Although *N. c.*-biomass could reduce Cr(VI) without the assistance of light energy, the reduction rate was low with the first-order rate constant less than 0.0010 min^{-1}, and about 46–80% of added Cr(VI) still present in the solution after 6 h of reaction (Fig. 1a). That is, 1–2.7 mg Cr(VI) was reduced by 1 g biomass in the

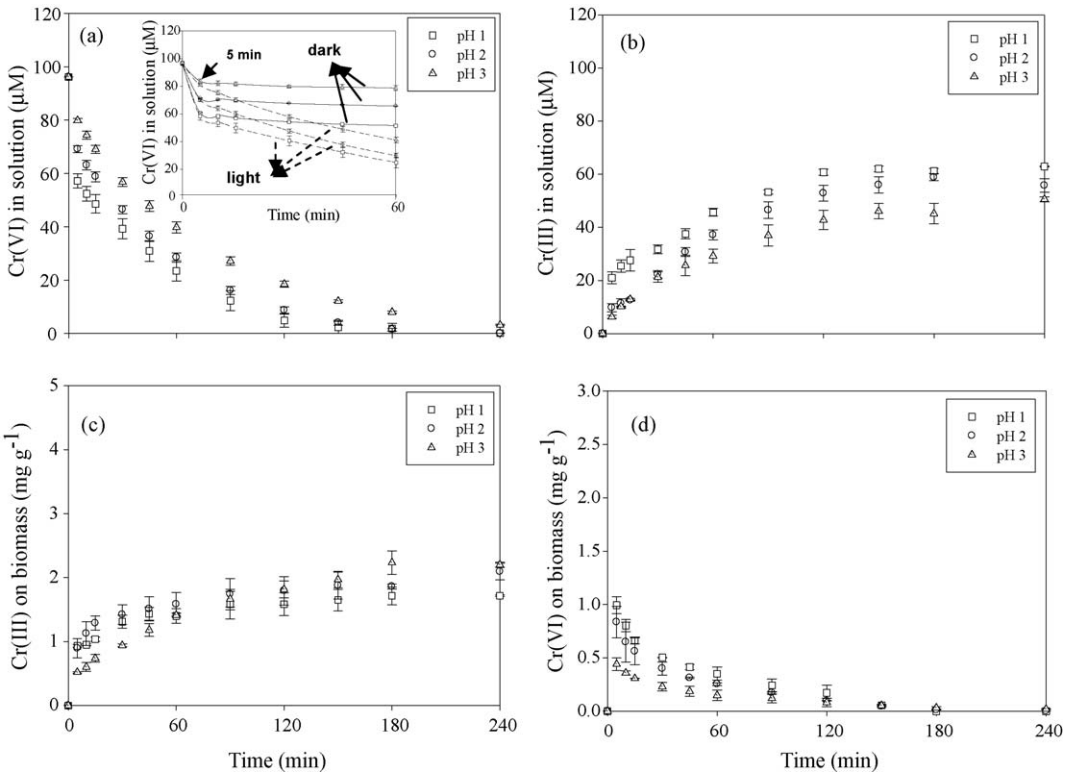

Fig. 2. Removal of 96.2 μM Cr(VI) by a dead *N. c.* fungal biomass (1 g L^{-1}) at pH 1–3 under light. The disappearances of Cr(VI) in solution and biomass are shown in (a and d), respectively. The formation of Cr(III) in solution and in biomass are shown in (b and c), respectively. Insert in (a) is a comparison of the changes of Cr(VI) concentration in solution in the dark and light.

absence of light at pH 1–3. Comparatively, a rapid decrease in Cr(VI) concentration was observed when light was available. The first-order rate constants increased from 0.0134 to 0.0231 min^{-1} with a decrease in pH from 3 to 1, and were significantly higher than the corresponding rate constants obtained without light (Table 1). With 96.2 μM of Cr(VI), complete reduction, which explained ca. 5 mg Cr(VI) reduction by 1 g biomass, was observed after 4 h irradiation (Fig. 2a). The reaction led to the formation of Cr(III) either released back to the solution (Fig. 2b) or bound to the biomass (Fig. 2c). During the first 5 min of reaction, the difference in Cr(VI) removal with light as compared to that in the dark was small; nonetheless, the differences increased with a prolonged reaction time (Fig. 2a, inserted figure). The enhancement of Cr(VI) reduction may be due to the absorption of light by biomass, resulting in a photo-induced oxidation of biomass and the formation of superoxide for Cr(VI) reduction (Eqs. (4)–(6)). In the presence of dissolved organic carbons, photo-induced the production of superoxide, a reductant for Cr(VI), had been previously reported [21]. Although superoxide measurement was not conduced in the study, the photo-reduction of Cr(VI) in a N_2-equilibrated atmosphere by biomass was indeed declined compared with the counterpart in a O_2-equilibrated atmosphere (data not shown). This result suggested that O_2 should be involved in the photo-reduction reaction.

$$\text{Biomass (in dissolved form)} + h\nu + O_2 \rightarrow \text{Biomass}^{\bullet+} + O_2^{\bullet-} \quad (4)$$

$$\text{Biomass}^{\bullet+} + O_2 \rightarrow \frac{\text{Biomass}^{\bullet+}}{CO_2} + O_2^{\bullet-} \quad (5)$$

$$\text{Cr(VI)} + 3\,O_2^{\bullet-} \rightarrow \text{Cr(III)} + 3O_2 \quad (6)$$

Even if photo-reduction of Cr(VI) was lower in an anaerobic environment, the overall Cr(VI) reduction was still higher than that in the dark. This was attributed to the direct reduction of Cr(VI) by excited biomass under illumination. That is, upon absorption of light, the production of excited state of biomass may be able to overcome the energy barrier for rapid Cr(VI) reduction (Eq. (7), Fig. 2d).

$$\text{Biomass} + h\nu \rightarrow \text{Biomass}^{*} + \text{Cr(VI)} \rightarrow \frac{\text{Biomass}_{\text{decomposed}}}{CO_2} + \text{Cr(III)} \quad (7)$$

In addition, photo-induced Cr(VI) reduction by the photolysis of H_2O molecules may contribute partially to the rapid disappearance of Cr(VI) (Eqs. (8)–(10)) [24].

$$HCrO_4^- + 7H^+ + 3e^- \rightarrow Cr^{3+} + 4H_2O \quad (8)$$

$$O_{2(g)} + 4H^+ + 4e^- \rightarrow 2H_2O \quad (9)$$

$$4HCrO_4^- + 16H^+ \rightarrow 4Cr^{3+} + 3O_{2(g)} + 10H_2O \quad (10)$$

where Eq. (10) was obtained by combining Eqs. (8) and (9).

3.3. Photo-reduction of Cr(VI) on N. c.-biomass as influenced by Fe(III)

Photo-reduction of Cr(VI) by biomass was further enhanced when 89.5 μM of Fe(III) were added (Fig. 3). For Cr(VI) reduction at pH 1, the reaction rates in the presence of light and light/Fe(III), increased 23-fold and 102-fold, respectively, compared to the rates in the dark (Table 1). The acceleration of Cr(VI) reduction in the presence of Fe(III) may be due to the photolysis of organic and inorganic Fe(III) complexes, resulting in the formation of Fe(II) for Cr(VI) reduction. However, compared with the amounts of Cr(VI) reduction with or without Fe(III) in the dark, both systems showed about 2.7 mg Cr(VI) reduction per 1 g biomass, which indicated that Fe(III) effects could be ignored while light was absent.

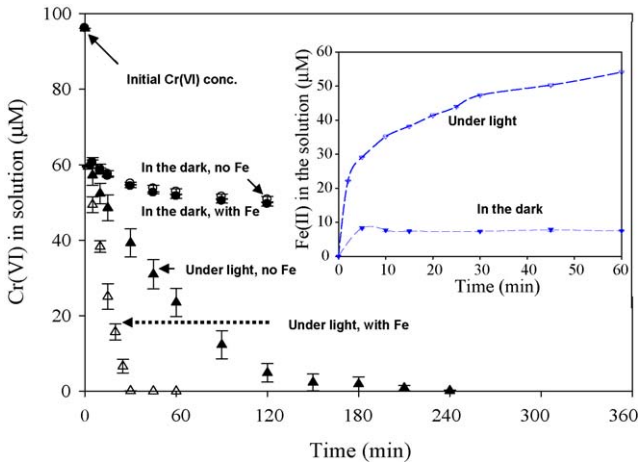

Fig. 3. Reduction of 96.2 μM Cr(VI) on 1 g L^{-1} *N. c.*-biomass in the presence or absence of 89.5 μM Fe(III) in the dark or light at pH 1. Insert shows that Fe(II) was produced upon the reduction of 89.5 μM Fe(III) by 1 g L^{-1} *N. c.*-biomass at pH 1.

In the presence of organics and Fe(III), a metal–organic complex may be formed through an ion-exchange reaction. Upon exposure of the complex to the light, a ligand-to-metal electron transfer would be initiated, leading to the productions of Fe(II) and biomass radicals (Eq. (11)) [25]. These intermediates and products then participate in subsequent Cr(VI) reduction as shown at Eqs. (5), (6) and (12).

$$\text{Fe(III)}-\text{Biomass} + h\nu \rightarrow \text{Biomass}^{\bullet+} + \text{Fe(II)} \quad (11)$$

$$HCrO_4^- + 3Fe^{2+} + 7H^+ \rightarrow Cr^{3+} + 3Fe^{3+} + 4H_2O \quad (12)$$

In the current system, photo-induced Fe(II) production was investigated in the absence of Cr(VI) due to the rapid reduction of Cr(VI) by Fe(II) at an acidic solution [26], and the result was shown in Fig. 3, inserted figure. A slight amount of Fe(II) (less than 10 μM) was produced in the dark; however, a significant increase in Fe(II) concentration was observed in the presence of light, reaching to 55 μM after 60 min of irradiation. The result supported the hypothetic pathway of photo-induced Fe(II) formation in the system with *N. c.*-biomass and Fe(III) (Eq. (11)).

As stated previously, Fe(II) production could also result from the photolysis of inorganic Fe(III) complexes. For instance, in the current system with Fe(III) and Cl^-, $FeCl^{2+}$ could be produced at pH 1. The photolysis of $FeCl^{2+}$ complex, forming Fe(II) and Cl radical, had been found a pathway for Cr(VI) reduction (Eq. (13)) [24].

$$FeCl^{2+} + H_2O \xrightarrow{h\nu} Fe^{2+} + \cdot Cl \quad (13)$$

Since Fe(II) was an excellent reductant for Cr(VI) in acidic solutions (Eq. (12)) [26], the photo-enhancement of Cr(VI) reduction with the addition of Fe(III) was expected.

Our previous study found that photo-induced Fe(II) production through Eq. (13) was about 9 μM [24], which was much lower than that obtained in the current system with biomass (Fig. 3, inserted figure). Therefore, Fe(III) should have formed a complex with the oxygen containing groups even if they were protonated at an acidic solution. Upon the exposure to the light, the electrons were transferred rapidly from ligands to Fe(III) and resulted in Fe(II) formation. Because the complexation constant of Fe(III) and *N. c.* biomass is unknown, the precise evaluation of the proportions of organic and inorganic Fe(III) complexes in the current system is impossible. Therefore, it was difficult to define the relative contribution of organic and inorganic Fe(III) complex to the enhancement of Cr(VI) photo-reduction.

With an increase of pH from 1 to 3, the first-order rate constant decreased from 0.0920 to 0.0235 min^{-1} (Table 1). Since the reduction of Cr(VI) by Fe(II) consumed protons (Eq. (12)), the increase in solution pH would definitely inhibit the redox reaction. With an increase of pH from 1 to 3, a portion of Fe^{3+} would be hydrolyzed, forming $Fe(OH)^{2+}$. Upon the absorption of light energy, the photolysis of $Fe(OH)^{2+}$ would lead to the production of OH radicals (Eq. (14)) [24]. However, the OH radicals may not inhibit Cr(VI) reduction through a back oxidation of Cr(III) to Cr(VI) (Eq. (15)) because the strong oxidants would be rapidly scavenged by biomass following their formations.

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + {}^{\bullet}OH \quad (14)$$

$$3{}^{\bullet}OH + Cr(III) \rightarrow Cr(VI) + 3OH^- \quad (15)$$

Thus we speculated that the decrease in Cr(VI) reduction on *N. c.*-biomass with an increase in pH in the presence of Fe(III) may be due to the low efficiency of ligand-to-metal electron transfer at a higher pH. This could be confirmed by rapid decrease in Fe(II) production on biomass at pH 3 (ca. 13 μM).

3.4. Spectroscopic studies

Elemental compositions of *N. c.*-biomass are listed in Table 2. The contents of N,C,H, and O composed of 96.4% (w/w) of biomass, and the rest may be attributed to phosphorus, sulfur, and other trace elements. The FTIR spectra of raw and Cr(VI)-treated *N. c.*-biomass were first examined to elucidate the mechanism of Cr(VI) removal by *N. c.*-biomass. To optimize the spectroscopic examinations of the changes in functional groups for Cr(VI)-loaded biomass, a higher concentration of Cr(VI) (1.92 mM) was added with the application of light energy. As shown in Fig. 4a, a broad absorption peak around 3250–3350 cm^{-1} indicated the stretching vibration of hydroxyl and amide groups of the biomass. The peaks at 2926 and 2858 cm^{-1} were assigned to $-CH_2$ and $-CH_3$ stretching in aliphatic structure, and those at 1737 and 1655 cm^{-1} to the –C=O stretching and asymmetric carboxylic stretching/or amide I band, respectively [13,27,28]. The absorption peak at 1550 cm^{-1} was attributed to amide II band, and 1241 cm^{-1} to symmetric stretching band of –C–O [15]. The strong peaks at 1152, 1077, and 1040 cm^{-1} were assigned to –C–O bands of polysaccharides(chitin)/glucan or characteristic P=O/P–OH absorption of glycoproteins [29–31]. Aminopolysaccharides and glycoproteins, which composed of chitin and glucan, respectively, are the major components of cell walls for filamentous fungi such as *Neurospora* [32].

After reacted with Cr(VI), four major changes of the functional groups on the biomass were observed in the corresponding FTIR spectra (Fig. 4b). The decreases in the intensities of the peaks at 2926 and 2858 cm^{-1}, corresponding to $-CH_2$ and $-CH_3$ stretching, indicated the loss of –CH groups on biomass upon the application of Cr(VI). The loss may result from a direct oxidation of CH groups by Cr(VI) or the release of CH groups from the oxidation of specific functional groups such as amide groups. The protonation of amide groups in an acidic solution [33], leading to the formation of positive charges followed by Cr(VI) adsorption, may be the potential trigger for the oxidation of amide groups and the release of –CH groups from

Table 2
Elemental compositions and point zero charge (PZC) of raw *N. c.*-biomass.

Sample	N[a] (%)	C (%)	H (%)	O (%)	PZC[b] (%)
N. c. raw	6.27 ± 0.02[c]	42.7 ± 0.01	5.97 ± 0.01	41.5 ± 0.04	6.55

[a] The percentage of N, C, H, and O were on the basis of weight (wt%).
[b] PZC of *N. c.* was determined by pH drift method as described by Lopez-Ramon et al. [38].
[c] Standard deviation.

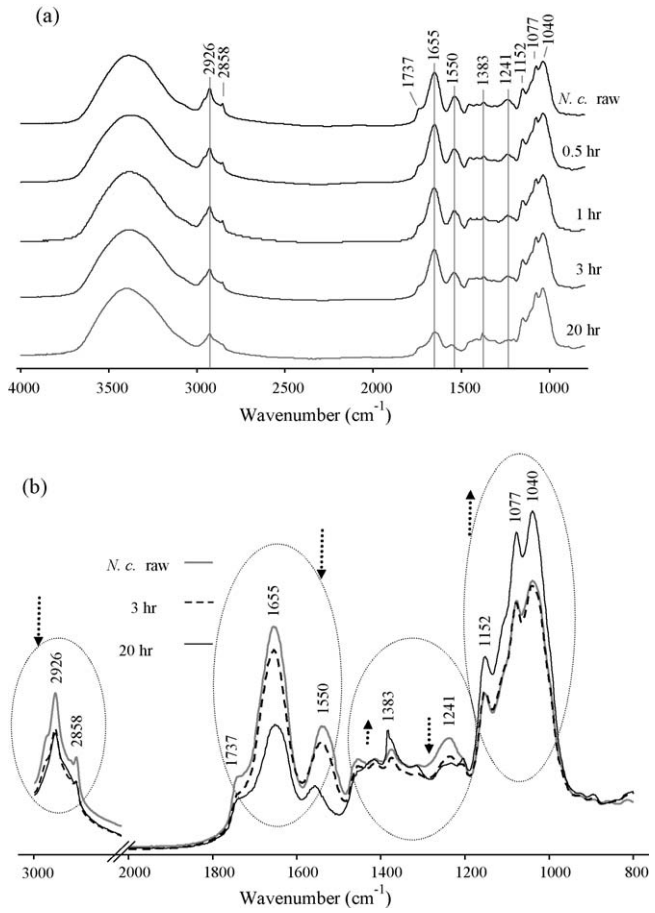

Fig. 4. FTIR spectra of (a) raw and Cr-loaded *N. c.*-biomass at different reaction times; (b) selected biomass in the region of 500–3000 cm^{-1}. The spectrum of raw *N. c.*-biomass was collected with biomass treated with 0.1 M HCl.

the biomass (Fig. 5). The derivatives of the oxidation of amide/–NH groups and the release of loosed bond organic carbons (i.e., DOC) may act as reductants for Cr(VI) in solution (Fig. 5). Accompanied by the oxidation of amide groups, the decrease in the absorption peaks at 1550 and 1655 cm^{-1}, which are N-related groups, was observed (Fig. 4). The decline of absorption peak at 1655 cm^{-1} may be also attributed to the oxidation of carboxyl groups (with a pK_a of 4.6 ± 0.1) [34] or –C=O of amide groups.

The decrease at 1737 cm^{-1} corresponded to the oxidation of carboxyl groups by Cr(VI) or the adsorption of Cr(III) on the carboxyl groups, leading to the shift of –C=O band to a lower frequency at 1639–1648 cm^{-1} [35]. In addition, the increase in the absorption peak at 1383 cm^{-1} may be due to the shift of 1241 cm^{-1}, indicating –C–O groups, upon Cr(III) binding [35]. Accompanied with the oxidation of amide and carboxyl groups by Cr(VI), the –C–O bands of polysaccharides (including chitin)/glucan or P=O/P–OH that is characteristic absorption of glycoproteins may condense on the biomass, resulting in an increase in absorption peaks in the region of 1040–152 cm^{-1} (Fig. 4b).

The Cr(VI)-loaded biomass was also investigated by NMR spectroscopy, and the results were shown in Fig. 6. ^{13}C CP-MAS NMR spectra of biomass correspond to aliphatic C (0–12 ppm), aromatic C (112–163 ppm), and carboxyl and carbonyl C (163–220 ppm) [36]. The peaks in the region of 50–12 ppm were assigned to 6 C on the repeated units of N-acetyl-β-D-glucosamine of chitin and glucan, the major components of cell walls of fungi (Fig. 6). With the addition of Cr(VI), the peaks in the region of 50–12 ppm were slightly decreased, which indicated that the cyclo-aliphatic C of chitin, glucan and their derivatives was persistent to

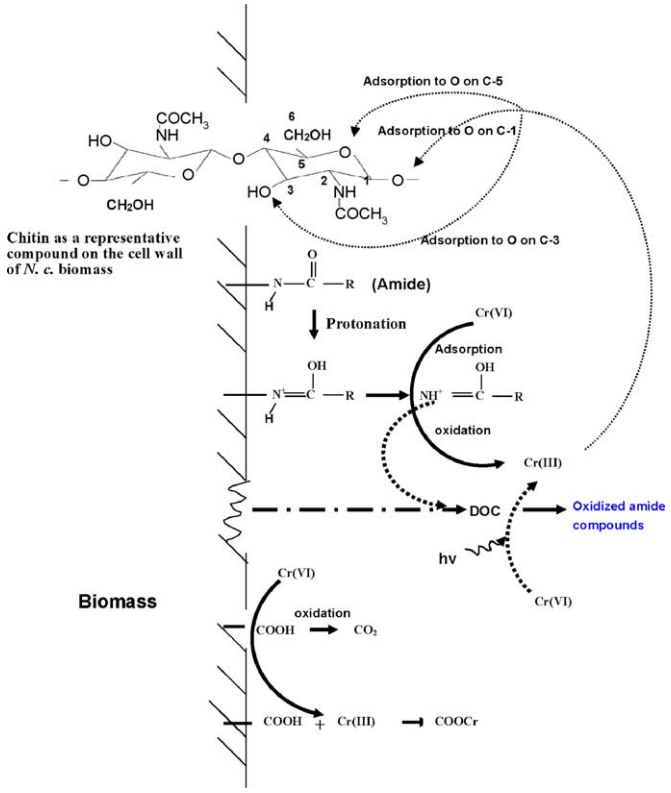

Fig. 5. Proposed model of Cr(VI) interactions with specific organic groups of the biomass.

the attack of Cr(VI). However, the peaks at 30.3 ppm and 173.2 ppm, representative of alky C and carboxyl C/or –C=O in amide groups, respectively, decreased significantly over the reaction time (Fig. 6). Accordingly, the –CH groups and –C=O groups, which derived partly from amide groups on biomass, may be involved in Cr(VI) reduction. The results are consistent with those of the corresponding FTIR spectra. However, the amide

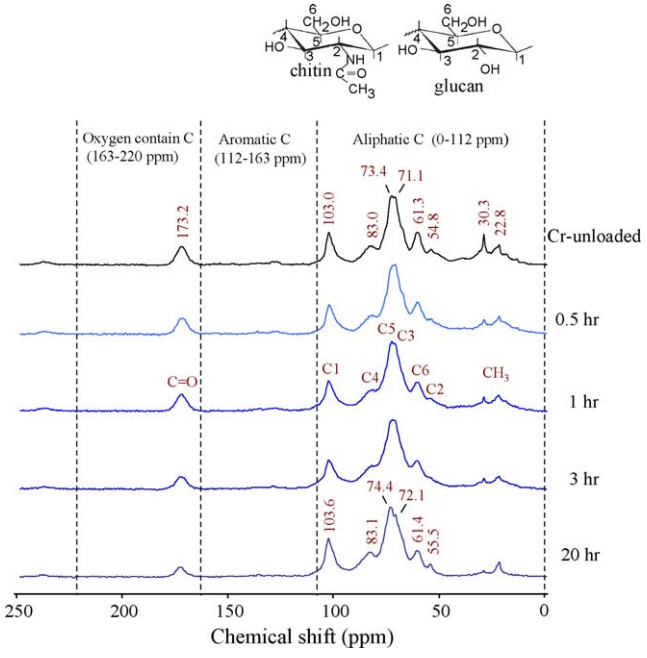

Fig. 6. NMR spectra of raw and Cr-loaded *N. c.*-biomass at different reaction times. The spectrum of raw *N. c.*-biomass was collected with biomass treated with 0.1 M HCl.

groups, which were susceptive to the attack by Cr(VI), could not refer to the acetamido groups (–NH(C═O)CH_3) of chitin or chitosan on *N. c.* biomass because the peak at 22.8 ppm, indicating –CH_3 on chitin, did not change significantly during the reaction (Fig. 6). Upon Cr(VI) reduction, a portion of the redox products of Cr(III) were bound on the oxygen attached to the C-1, C-3, and C-5 of chitin/glucan, leading to a slight increase in chemical shifts of NMR spectra (Fig. 6) [37].

4. Conclusions

The adsorption and reduction of Cr(VI) on *N. crassa* were first investigated in the dark. The rate of Cr(VI) removal by dead fungal biomass followed the first-order kinetics, and it increased with decreasing pH and the application of light energy. Cr(VI) removal was mainly attributed to the reduction reaction due to the appearance of Cr(III), which was not initially present. The protonation of specific functional groups such as N-containing groups on the surfaces of biomass, leading to an increase in positive charges, was responsible for the increase in the adsorption and subsequent reduction of Cr(VI) with the decrease at solution pH. Photo-induced the formation of superoxide or excited state of biomass may overcome the energy barrier for the rapid Cr(VI) reduction. Cr(VI) reduction was further enhanced with the addition of ferric ion, a strong complexation ligand for organics. Upon the absorption of light energy, the photolysis of Fe–organic complexes, resulting in the formation of Fe(II) may provide an additional pathway for accelerating Cr(VI) reduction. Spectroscopic analysis indicated that cyclo-carbons of chitin, glucan, and their derivatives on *N. c.*-biomass may not participate initially in Cr(VI) reduction. It is carboxyl or N-containing groups such as amide groups or –NH in *N. c.*-biomass initiated mainly the adsorption and subsequent Cr(VI) reduction. By examining the results of Cr(VI)-loaded biomass obtained in the dark and under light, no spectroscopic differences were observed, which suggested that light could only enhance Cr(VI) reduction on specific organic groups such as carboxyl and amide groups. Since the fungi containing the carboxyl, –NH, and amide groups contributed mainly to Cr(VI) reduction, these types of fungi may act preferentially as reductants in a sunlit water body.

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